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Research article

Bactericidal activity of *Piper betle* L. extract against antibiotic resistant *Salmonella* spp. isolated from pig farms in Southern Thailand

Ratchadaporn Boripun[1,6], Panida Paopradit[2], Jindarha Prampramote[3], Ruethai Narinthorn[1], Sutsiree Intongead[1], Suthinee Sangkanu[4], Tanapat Narongrit[5] and Watcharapong Mitsuwan[1,6,7,*]

[1]*Akkhraratchakumari Veterinary College, Walailak University, Nakhon Si Thammarat 80160, Thailand*
[2]*Faculty of Agriculture, Princess of Naradhiwas University, Narathiwas 96000, Thailand*
[3]*School of Agricultural Technology and food Industry Walailak University, Nakhon Si Thammarat 80160, Thailand*
[4]*School of Allied Health Sciences, Southeast Asia Water Team (SEA Water Team) and World Union for Herbal Drug Discovery (WUHeDD), Walailak University, Nakhon Si Thammarat 80160, Thailand*
[5]*Division of Biological Science, Faculty of sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[6]*One Health Research Center, Walailak University, Nakhon Si Thammarat 80160, Thailand*
[7]*Center of Excellence in Innovation of Essential Oil, Walailak University, Nakhon Si Thammarat 80160, Thailand*

Abstract

Piper betle L. leaves have traditionally been used to treat various infectious diseases and to possess a wide spectrum of pharmacological effects. This study aimed to determine the antibacterial activity of the *Piper betle* leaf extract against antibiotic resistant *Salmonella* spp. isolated from pig farms located in Southern Thailand. Of this, 12 *Salmonella* spp. isolates were isolated from 24 pig fecal samples from 24 pig farms. The isolates were resistant to ampicillin (91.67%), penicillin (91.67%), tetracycline (81.81%), and doxycycline (81.81%). Antibacterial activity of the *Piper betle* ethanolic leaf extract against *Salmonella* spp. was carried out by disc diffusion assays, followed by Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) determination, as well as Time kill study. *Piper betle* extract exhibited antibacterial activity against all the isolates and *S.* Typhimurium with the inhibition zone ranged from 15.11 ± 0.34 to 20.30 ± 0.50 mm as observed by disc diffusion assay. The extract showed bactericidal activity against the isolates with the MIC and MBC values ranging from 0.5-1.0 mg/mL. Furthermore, the extract at 4 × MIC showed the killing activity with the reduction of the pathogen at least 3 logs within 8 h. The information suggests potential medicinal benefits of the *Piper betle* leaf extract to inhibit the growth of antibiotic resistant *Salmonella* spp. isolated from pig farms.

Keywords: Antibacterial activity, Antibiotic resistance, Pig farms, *Piper betle*, *Salmonella* spp.

Corresponding author: Watcharapong Mitsuwan, Akkhraratchakumari Veterinary College, Walailak University Nakhon Si Thammarat 80160, Thailand. Email: 1234_k@hotmail.co.th, watcharapong.mi@wu.ac.th.

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INTRODUCTION

Salmonella spp. is a Gram-negative bacterium that can be found in the intestinal tract in various species of animals including pigs. The genus *Salmonella* contains two species including *Salmonella bongori* and *Salmonella enterica* that is divided into six subspecies that include over 2,600 serotypes (Gal-Mor et al., 2014). S. enterica subspecies enterica serovar Typhimurium (*S.* Typhimurium) is one of the most common pathogens that cause inflammation and necrosis of the small and large intestines, resulting in diarrhea (Meurens et al., 2009). Pigs in all ages are susceptible to the pathogen especially the weaned and growing-finishing animals (Meurens et al., 2009). *Salmonella* produces necrotizing enterocolitis that associated with septicemia. Hence, *Salmonella* spp. is an important pathogen that causes economic losses in pig production worldwide. In fact, *Salmonella* can infect sequentially and transiently by various serotypes that can cause food poisoning in humans. The bacterium is also zoonotic human pathogen associated with fresh produce related foodborne illnesses in human (Fakruddin et al., 2017).

Antimicrobial resistant *Salmonella* spp. is of increasing concern in the meat industry as well as in human health. The previous studies have been shown that the *Salmonella* spp. isolates from pork retail outlets (Holohan et al., 2022), poultry (Vidayanti et al., 2021), and patients (Wu et al., 2021) demonstrate high prevalence of antibiotic resistance. Importantly, the occurrence of multi-drug resistant *Salmonella* spp. isolates has gained more attention worldwide (Lauteri et al., 2021). Furthermore, the treatment of the infections is becoming difficult due to the increasing antibiotic resistance of the clinically bacterial isolates.

In attempt to overcome the multi-drug resistant *Salmonella* spp., natural products based on their secondary metabolites have been used as an alternative treatment against the pathogen (Naz et al., 2022). The aqueous extracts of oregano, thyme, calendula, and basil exhibited bactericidal activity against S. Typhimurium(Gavriil et al., 2021). In the present study, we focused on *Piper betle* L. leaf extract, known as a betel vine. The plant species, belongs to the Piperaceae family, has been used in traditional medicine for the treatment of several infectious diseases (Pecková et al., 2018). Recently, antibacterial activity of *Piper betle* leaf extract against Avian pathogenic *Escherichia coli* (APEC) has been previously reported (Kulnanan et al., 2022). Furthermore, the extract inhibited the bacterial adhesion, resulted to reduce the biofilm formation in APEC (Kulnanan et al., 2022). Antibacterial activity of *Piper betle* ethanolic leaf extract against other pathogens including *Staphylococcus aureus*, *P. aeruginosa*, and *Salmonella* spp. has also been documented (Ermawati et al., 2021).

To the best of our knowledge, no study on this local plant against *Salmonella* spp. isolated from pig farms has been scientifically documented. Therefore, the objective of this study was to investigate the antimicrobial activity of *Piper betle* ethanolic leaf extract against *Salmonella* spp. isolated from pig farms. The antibacterial activity was determined using disc diffusion assay, broth microdilution assay, and time kill kinetic study.

MATERIALS AND METHODS

Samples collection

This article does not contain any studies with human participants or animals performed by any of the authors. A total of fecal 24 samples of health pig were collected from 24 pig farms during a 2-months period (October to November 2021) in Tha Sala district, Nakhon Si Thammarat province, Thailand. After collection, these samples were kept in sterile containers, preserved at 4°C, and performed the isolation of the bacteria within 24 h.

Isolation and Identification of Salmonella spp. from pig farms

All samples were cultured by standard culture methods following the 2002 ISO *Salmonella* rule 6579 (ISO, 2007). Briefly, 25 g of each sample was suspended in 225 mL of 0.1% buffered peptone water (BPW) (Oxoid, Hampshire, UK), and incubated at 37°C for 24 h. These microbial enrichments were streaked onto modified semisolid Rappaport-Vassiliadis medium (MSRV) (Oxoid, UK) and incubated at 41.5°C for 24 h. A loopful of microorganisms taken from the edge of the MSRV colony was inoculated onto Xylose-lysine-deoxycholate agar (XLD) (Oxoid, UK), at 37°C for 24 h. Subsequently, suspected colonies of *Salmonella* spp. (dark) were picked from each selective plate and streaked onto Tryptic Soy Agar (TSA) (Difco, Claix, France) and incubated at 37°C for 24 h., followed by biochemical identification using Vitek2 (bioMérieux, Marcy 10 Etoile, France).

Antibiotic susceptibility test

Antibiotic susceptibility profiles of *Salmonella* spp. isolated from pig farms and S. Typhimurium (the reference strain) were carried out by disc diffusion assay according to CLSI guidelines as described (CLSI, 2019). Briefly, 3-5 colonies of the bacteria cultured on Mueller Hinton agar (MHA) (Difco, Claix, France) were suspended in PBS to adjust the density using McFarland No. 0.5 standard. The samples were then spread on the MHA plates. Antibiotic discs recommended for the treatment of *Salmonella* infection including 10 mg of ampicillin (Amp) (Oxoid, Hampshire, UK), tetracycline (Tet) (Oxoid, Hampshire, UK), doxycycline (Dox) (Oxoid, Hampshire, UK), enrofloxacin (Enr) (Oxoid, Hampshire, UK), gentamicin (Gen) (Oxoid, Hampshire, UK), trimethoprim (Tri) (Oxoid, Hampshire, UK), 30 mg each of ceftazidime (Caz) (Oxoid, Hampshire, UK), and cefotaxime (Ctx) (Oxoid, Hampshire, UK) were added on the plates. The samples were then incubated at 37°C for 18-20 h. The inhibition zone was measured and analyze according to CLSI 2019 guidelines. *S.* Typhimuriumwas used as the reference strains. Multidrug resistance (MDR) is defined as antimicrobial resistance shown by the isolate to at least one antimicrobial drug in three or more antimicrobial categories.

Preparation of plant extracts

Fresh and mature leaves of *Piper betle* were collected from Phatthalung province, Southern, Thailand. Identification of the plant was carried out by Associate Professor Dr. Chatchai Kanlayanapaphon, Department of Biology, School of Sciences, Walailak University. The morphological characteristics of the plant were followed by the literature of Flora of Java volume 1 (Erwiyani et al., 2017). To prepare the *Piper betle* leaf extracts, the leaves were washed with running distilled water to remove all contaminants. The plants were then cut into small pieces and dried at room 37 °C for 2 hours. After that, they were dried in an oven at 40 °C for 3 days. The plants were shade-dried and powdered by using a dry blender and packed in an airtight container for storage until analysis. Fifty grams of dry powder was weighed and allowed to soak in 200 mL of 95% ethanol for 7 days at room temperature as previously described (Kulnanan et al., 2022; Mitsuwan et al., 2020). The suspension was filtered and evaporated under reduced pressure using a vacuum evaporator to obtain the extracts. The extracts were air-dried at room temperature to remove the rest of the solvent, and to maintain the dry weight of the plant powder extracts. Then, the extracts were balanced daily until the weight stable. The extracts were dissolved in 100% dimethyl sulfoxide (DMSO) and stored at 4°C for further use.

Disc diffusion assay

The antibacterial activity of *Piper betle* leaf extract was evaluated by disc diffusion method (Jaber et al., 2021; Kulnanan et al., 2022). The extract was prepared at 200 mg/ml in 100% DMSO. Then, 12.5 µL of the stock solution was loaded into six millimeters of sterile filter paper discs to obtain a concentration of 2.5 mg/disc. Cultures of the antibiotic resistant *Salmonella* spp. isolated from pig farms were adjusted to the McFarland No. 0.5 standard and swab on Mueller-Hinton agar (MHA) (Difco, Claix, France) plates. The discs were then placed onto the surface of bacterial cultured plates and incubated at 37°C for 18 h. *S*. Typhimurium was included as the reference strain. Gentamicin and Penicillin G were used as positive controls. 1% DMSO was taken as the negative control. The diameter of the inhibition zones (mm) was measured. Values are the mean of triplicate. The data was presented as mean±SD.

Determination of Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC)

The antibacterial activity of the *Piper betle* leaf extract against antibiotics resistant *Salmonella* spp. isolated from pig farms was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) as previously demonstrated (Jaber et al., 2021; Mitsuwan et al., 2020). Briefly, the leaf extracts were diluted in a 96-well microtitre plate to the final concentration ranging from 2.0 to 0.125 mg/mL in Mueller-Hinton broth (MHB) (Difco, Claix, France). One hundred microliter of the bacterialsuspensions (final concentration 10^6 CFU/mL) in MHB of each selected bacteria was inoculated to well and incubated at 37°C for 18 h. The reference strain, *S*. Typhimurium, was included in each batch of MIC testing to

corroborate validity of the results. Ceftriaxone and 1%DMSO were used as the positive and negative control, respectively. Then, resazurin (0.03%) (Thermo Fisher Scientific, Lancashire, UK) was added to the wells for the observation of color changes. The MIC was defined as the lowest concentration that completely inhibited the growth of bacteria that present a blue color (Pereira et al., 2021). The MBC was performed with the extract that gave significant MIC values, by direct streaking the content of wells on TSA plates. Experiment was repeated thrice for authentication of the data.

Time-kill study

Time-kill kinetic study of the *Piper betle* extract against a representative multidrug resistant *Salmonella* spp.WU22006 was investigated. The concentrations of *Piper betle* extract at 1, 2, and 4 times of MIC were prepared and incorporated with the test bacteria which have a final inoculum density of approximately 106 CFU/mL. One percent DMSO was used as a negative control. After incubation at 37ºC, 100 µL of the samples were taken at 0, 2, 4, 8, 12, 16, and 24 h to dilute in sterile phosphate buffered saline (PBS) and drop onto TSA plate in order to count the viable colony number. The plates were incubated at 37°C for 24 h. The experiment was done in three replicates and the results were calculated as mean log numbers of microorganisms ± standard deviation.

Statistical analysis

The data were analyzed using the statistical package software (SPSS Inc.Chicago, IL, USA).The results were presented as mean±SD. Furthermore, the two-tailed unpaired Student's t-test was also analyzed. Differences were considered significant at $P<0.05$. All experiments were performed in triplicate.

RESULTS

Isolation and antibiotic susceptibility of *Salmonella* spp. from pig farms

A total of 24 fecal samples from different pig farms located in Nakhon Si Thammarat province was collected in this study. Of this, 12 *Salmonella* spp. isolates were isolated from these samples (Table 1). Furthermore, antibiotic susceptibility of the isolates was determined by disc diffusion assay according to CLSI. Ten antibiotics were used to carried out the antimicrobial susceptibility according to the Gram-negative panel of antibiotics recommended by the CLSI and drug used to treat the infection caused by *Salmonella* spp. As shown in Table 1, the isolates were sensitive to gentamicin, enrofloxacin, cefotaxime, and ceftazidime. However, the isolates were resistant to ampicillin (91.67%), penicillin (91.67%), tetracycline (81.81%), and doxycycline (81.81%).

Table 1 Antimicrobial susceptibility pattern of *Salmonella* spp. isolated from pig farms

Isolates	Antibiotic susceptibility	
	Resistance	Sensitive
WU22001	Amp-Pen-Tet-Dox	Gen-Tri-Enr-Ctx-Caz
WU22002	Amp-Pen-Tet-Dox	Gen-Tri-Enr-Ctx-Caz
WU22003	Amp-Pen-Tet-Dox	Gen-Tri-Enr-Ctx-Caz
WU22004	Amp-Pen	Gen-Tri-Enr-Ctx-Caz-Tet-Dox
WU22005	Amp-Pen	Gen-Tri-Enr-Ctx-Caz-Tet-Dox
WU22006[a]	Amp-Pen-Tet-Dox-Tri	Gen-Enr-Ctx-Caz
WU22007[a]	Amp-Pen-Tet-Dox-Tri	Gen-Enr-Ctx-Caz
WU22008	Amp-Pen-Tet-Dox	Gen-Tri-Enr-Ctx-Caz
WU22009	Amp-Pen-Tet-Dox	Gen-Tri-Enr-Ctx-Caz
WU22010	Amp-Pen-Tet-Dox	Gen-Tri-Enr-Ctx-Caz
WU22011[a]	Amp-Pen-Tet-Dox-Tri	Gen-Enr-Ctx-Caz
WU22012	-	Gen-Enr-Ctx-Caz-Amp-Pen-Tet-Dox-Tri
S. Typhimurium[a]	Amp-Pen-Tet-Dox-Tri	Gen-Enr-Ctx-Caz

[a], multi-drug resistance
Amp; ampicillin, Pen; penicillin, Tet; tetracycline, Dox; doxycycline, Tri; trimethoprim, Gen; gentamicin, Enr; enrofloxacin, Ctx; cefotaxime, Caz, ceftazidime

Preliminary screening of antibacterial activity of Piper betle extract against *Salmonella* spp.

In order to investigate antimicrobial activity of the extract against the isolates of *Salmonella* spp., the disc diffusion assay was used at a concentration of 2.5 mg/disc. As shown in Table 2 and Figure 1, *Piper betle* extract significantly exhibited antibacterial activity against all the isolates and *S.* Typhimurium, compared with the negative control (1%DMSO). The zone of inhibition of the extract against the isolates ranged from 15.11 ± 0.34 to 18.48 ± 0.40 mm. In addition, the inhibition zone of the extract against the reference strain, *S.* Typhimurium, was 20.30±0.50 mm. Gentamicin and penicillin G were included as a positive control. All the clinical isolates were found to be sensitive to gentamicin.

Table 2 Antibacterial activity of the *Piper betle* leaf extract on *Salmonella* spp. isolated from pig farms by disc diffusion assay

Isolates	Inhibition zone (mm)	Antibiotic susceptibility	
		Gentamicin	Penicillin
WU22001	15.81 ± 0.45	S	R
WU22002	17.13 ± 0.49	S	R
WU22003	17.95 ± 0.37	S	R
WU22004	18.48 ± 0.40	S	R
WU22005	17.34 ± 0.25	S	R
WU22006	16.84 ± 0.53	S	R
WU22007	15.54 ± 0.57	S	R
WU22008	16.32 ± 0.25	S	R
WU22009	17.36 ± 0.25	S	R
WU22010	15.11 ± 0.34	S	R
WU22011	16.68 ± 0.97	S	R
WU22012	17.91 ± 0.79	S	S
S. Typhimurium	20.30 ± 0.50	S	R

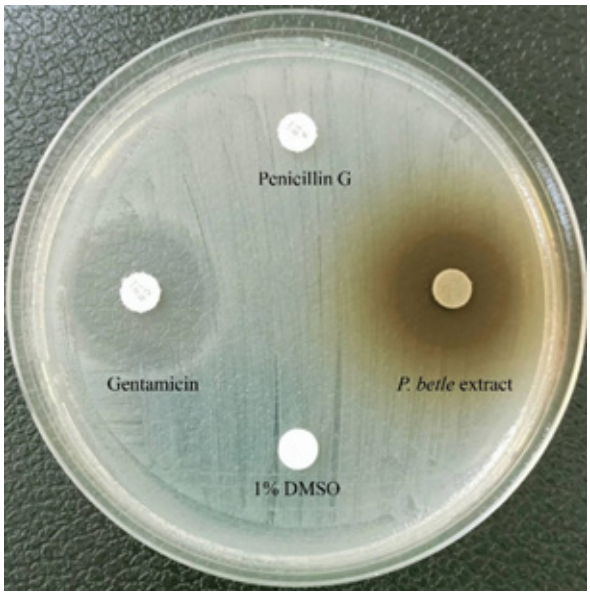

Figure 1 Activity of *Piper betle* extracts against a representatively isolate *Salmonella* sp. WU22006 as measured by disc diffusion assay. Gentamicin and penicillin were used as positive controls, while DMSO was included as a negative control. Inhibitory activity was presented as zones of inhibition.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *Piper betle* against *Salmonella* spp.

The MIC and MBC values of *Piper betle* extract against 12 *Salmonella* spp. isolates and *S.* Typhimurium were investigated by a broth microdilution assay according to CLSI. The results revealed that the *Piper betle* leaf extracts showed strong antibacterial activity against the isolates with the MIC and MBC values ranging from 0.5-1.0 mg/mL (Table 3). It was observed that the MIC and MBC values of the extract against *S.* TyphimuriumWU22001 were in the same range as those of the tested the *Salmonella* spp. isolates.

Table 3 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *Piper betle* leaf extract on *Salmonella* spp. isolated from pig farms

Isolates	Antibacterial activity (mg/ml)			
	Piper betle extract		Gentamicin	
	MIC	MBC	MIC	MBC
WU22001	1.0	1.0	0.00025	0.00025
WU22002	1.0	1.0	0.00050	0.00050
WU22003	1.0	1.0	0.00025	0.00050
WU22004	1.0	1.0	0.00025	0.00025
WU22005	1.0	1.0	0.00050	0.00050
WU22006	0.5	1.0	0.00050	0.00200
WU22007	0.5	0.5	0.00100	0.00200
WU22008	1.0	1.0	0.00050	0.00050
WU22009	1.0	1.0	0.00050	0.00200
WU22010	1.0	1.0	0.00025	0.00025
WU22011	1.0	1.0	0.00100	0.00100
WU22012	1.0	1.0	0.00025	0.00050
S. Typhimurium	0.5	0.5	0.00100	0.00100

Time kill study

In order to confirm antibacterial effectiveness of *Piper betle* extract against *Salmonella* spp., a time kill curve of the bacteria cultured in the medium plus the extracts at different concentrations based on the MIC value was determined. The isolate WU22006 was used as a representative due to its antibacterial resistant profile as multi-drug resistance. It was found that the extracts showed the antibacterial activity with a concentration dependent manner, resulting in the reduction of the viable cells of the isolate (Figure 2). The extract at 4×MIC showed the killing activity with the reduction of the pathogen at least 3 logs within 8 h. In addition, the viability of the isolates after treatment with 2×MIC of the extracts decreased by 3 logs in 12 h. However, it was found that the regrowth of these isolates was observed after treatment with the extracts at 1×MIC.

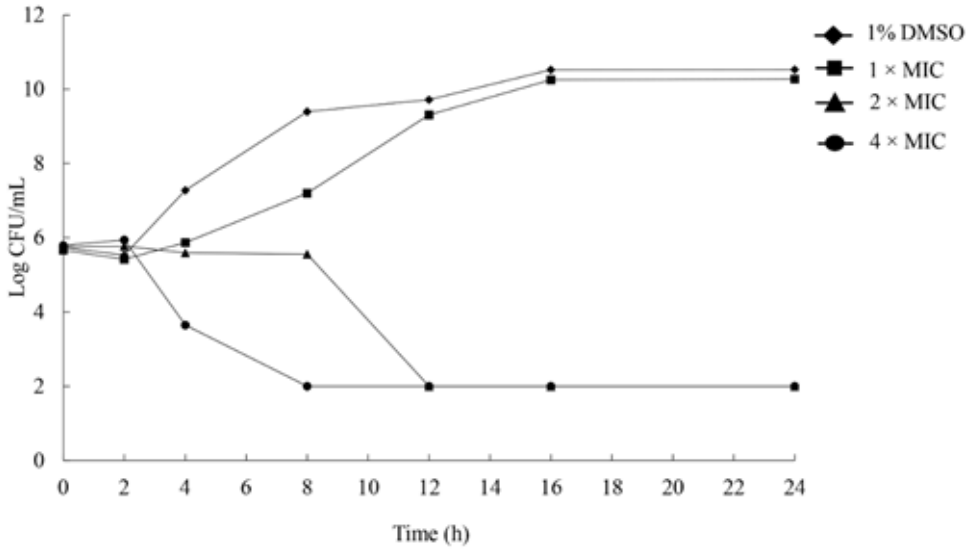

Figure 2 Time-kill curves of *Salmonella* sp. WU22006 after treatment with *Piper betle* extract. The pathogen was treated with *Piper betle* extract at different concentrations including 4×MIC, 2×MIC, 1×MIC. One percent DMSO was used as a negative control.

DISCUSSION

*Salmonella*e spp. including *S.* Typhimurium cause necrotic inflammation of the small and large intestines, resulting in diarrhea in pigs (Meurens et al., 2009). The pathogen is one of the zoonotic and foodborne pathogens that transmitted by consumption of raw pork or contaminated foods. Furthermore, pig feces have widely been used as natural fertilizers for plant agriculture and contributed as one of the possible sources of transmissions to human. It has been reported that clinical isolates of *Salmonella* spp. was naturally resistant to ampicillin, chloramphenicol, and sulphamethoxazole/trimethoprim (Gong et al., 2022). However, some study demonstrated that the clinical isolates of *Salmonella* spp. did not show 100% of the antibiotic resistance, which the data showed resistance to tetracycline (89.29%), followed by ampicillin (78.57%) and sulfamethoxazole-trimethoprim (71.43%) (Tadee et al. 2021). In this study, *Salmonella* spp. was isolated from pig feces and most of the isolates were resistant to ampicillin, penicillin, tetracycline, doxycycline, and trimethoprim. In this study showed that 3 isolates of *Salmonella* spp. were multi-drug resistance. Our finding is interestingly in agreement with a previous study reported on antibiotic resistant pattern of *Salmonella* strains isolated from local organic pig farming in northern Thailand (Tadee et al., 2021). To support this, the bacterium contains β-lactamases inducing genes including blaTEM, blaCTX-M, blaOXA, and blaCMY-2 tends to promote the resistance to β-lactam antibiotics including ampicillin and penicillin (Qin et al., 2022). Also, the most prominent resistance genes were those conferring resistance against tetracyclines as *tetA*, *tetB*, *tetD*, and *tetM* (Qin et al., 2022). Thus, many studies have long been focused on new drugs or compounds which have an ability to inhibit the antibiotic resistant bacteria.

Our study showed the bactericidal activity of *Piper betle* leaf extract against *Salmonella* spp. isolated from pig farms. The extract showed a bactericidal activity against *Salmonella* spp. and *S*. Typhimurium isolates due to the MBC/MIC ratio of the extracts against pathogens (Kulnanan et al., 2022). Recently, the antibacterial activity of *Piper betle* extract has been reported to inhibit the bacterial adhesion resulting to the reduction of biofilm formation in APEC (Kulnanan et al., 2022). In addition, the growth inhibition of methicillin-resistant *Staphylococcus aureus*, ESβL *Klebsiella pneumoniae*, ESβL *Pseudomonas aeruginosa*, and ESβL E. coli by *Piper betle* leaf extract has been reported (Valle et al., 2015). Interestingly, the reduction of *Salmonella* sp. WU22006 viability was observed at 4-8 h, as similarly shown in APEC CH09 (Kulnanan et al., 2022). The previous study investigated the antibacterial activity of *Piper betle L*. leaf extract on inhibiting *Staphylococcus aureus* in conjunctivitis patient (Lubis et al., 2020). Many studies have informed that the *Piper betle* extract also shows potential in wound healing and anti-aging treatment (Mukherjee et al., 2011). However, those studies shown the safety of this extract for using in human. In addition, *Piper betle* extract showed non-toxicity in mice administered daily for 2 weeks (Choudhary and Kale, 2002). Furthermore, the previous study demonstrated the non-toxicity of the ethanol extract of betel leaves on normal human dermal fibroblasts (Valle et al., 2016).

The leaf extract of *Piper betle* contains a variety of chemical substances including phenol, flavonoid, and tannin (Taukoorah et al., 2016) that contribute to its therapeutic potential. The inhibition of the bacterial cell membrane function and energy metabolism was speculated as the possible modes of action in flavonoids against the pathogens (Cushnie and Lamb, 2005). The phenolic compounds denatured the bacterial cell proteins and inhibited bacterial cell multiplication (Cowan et al., 1999). In addition, pure compounds of eugenol, phytol, 4-chromanol, hydroxychavicol, carvacrol, chavicol, chavibetol, and allylpyrocatechols 1 have been isolated from the leaf extracts of this plant species and its essential oil (Nayaka et al., 2021). Recently, hydroxychavicol was found to be the major compound of *Piper betle* leaf extract detected by GC-MS analysis and reported on the abnormal morphology (e.g., dried cell shape with long cell formation without septum) of APEC after treatment with this extract (Kulnanan et al., 2022). It has been documented that hydroxychavicol induces the bacterial cell death by DNA damage, ROS generation, and inhibition of the cell division (Singh et al., 2018). In addition, the pure compound downregulated the expression of SulA, a protein under the control of SOS response (Singh et al., 2018), resulting in the exhibition of long cell shape without FtsZ ring formation (Mukherjee et al., 1998). This may be one of the possible mechanisms of action of *Piper betle* extract against bacteria. Although, the killing activity of the extract was lower than the antibiotic. However, other pharmacological effects of the plant extract including anti-inflammatory activity that could be used as an alternative agent against the infection.

Limitations of this study are the identification of the serovar of *Salmonella* spp., and the detection of antibiotic resistant genes related to the antibiotic resistant patterns. More comprehensive studies on antibacterial activities of *Piper betle* leaf pure compounds against the pathogens should be carried out. Subsequently, the antibacterial mechanisms of the extract and its

pure compounds against *Salmonella* spp. should be investigated. Future study should also be determined in vivo/ex vivo to provide insights on the mechanism of *Piper betle* leaf extract and its pure compounds against *Salmonella* spp. found in animals.

CONCLUSIONS

Bactericidal activity of *Piper betle* extract against antibiotic resistant *Salmonella* spp. isolated from pig farms was presented in this study. The bactericidal activity of *Piper betle* extract with the MIC and MBC ranged from 0.5-1.0 mg/mL against the isolates of *Salmonella* spp. as well as *S.* Typhimurium. Further, *Piper betle* extract at 4×MIC showed the killing activity with the reduction of the isolates at least 3 logs within 8 h. The information suggested *Piper betle* leaf extract inhibited the growth of *Salmonella* spp. isolated from pig farms which can provide as potential medicinal benefits to farm animals in the future.

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AUTHOR CONTRIBUTIONS

WM, RB, PP, and JP conceived and designed the experiments. WM, RB, RN, SI, SS, JP and TN performed the experiments. WM, RB, JP, and PP analyzed and interpreted the data. WM analyzed statistical analysis. WM, RB, JP, and PP wrote the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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